The impact of vernalization requirement, photoperiod sensitivity and earliness per se on grain protein content of bread wheat (*Triticum aestivum* L.)

Markus Herndl · Jeffrey W. White · Simone Graeff · Wilhelm Claupein

Abstract In wheat, a shorter pre-anthesis phase is often associated with increased grain protein content (GPC) but decreased grain yield. Cultivar differences in pre-anthesis development are mainly determined by vernalization requirement, photoperiod sensitivity and earliness per se. This research examines whether cultivar differences in these traits affect GPC, especially whether the three traits can partially explain genotype × environment interactions for GPC. Twenty-four winter wheat and five spring wheat cultivars selected from International Winter Wheat Performance Nursery (IWWPN) trials and 12 winter wheats tested over 2 years in Germany were characterized using the CSM-Cropsim-CERES-Wheat model. The model parameter P1V specifies the cultivar vernalization requirement, P1D the photoperiod response, and P_{123} earliness per se. Covariance analyses of the IWWPN dataset indicated that about 7% of variation in GPC was explained by cultivar, with another 7% attributable to interactions of cultivar with region, site and year. P1V, P1D and P_{123} all influenced GPC, but their effects varied with region, site and year. For example, for two regions, the effect of P1V on GPC decreased with latitude. Path analyses using the data from Germany confirmed that GPC increased with earlier anthesis, which was influenced by P1D and P_{123}. Lack of an effect of P1V at this location presumably was due to all cultivars being completely vernalized. The results indicate that efforts to improve GPC could target the three traits to specific populations of environments, which should reduce the large influence of environment on GPC.

Keywords Grain protein content · Phenology · Earliness per se · Photoperiod · Vernalization · Modeling

Abbreviations
GPC   Grain protein content
P_{123} Earliness factor based on model parameters
NNFI   Non-normed fit index

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Introduction

Grain protein concentration (GPC) is a key determinant of grain quality in bread wheat, but improvement of GPC is challenging due to large effects of genotype, environment and their interactions (G × E). The relative importance of these factors varies among experiments and environments (e.g. Cox et al. 1985b; Rao et al. 1993). Baenziger et al. (1985) found that in a set of 22 soft and two hard wheat cultivars, tested in

12 environments, there was a 99 times higher variance component of environment than for cultivar. The effect of environment makes it difficult for breeders to test the influence of single traits on GPC, especially if effects of G × E are large. Major environmental factors influencing GPC include air temperature, soil nitrogen levels, water availability during grain filling, as well as post-anthesis light intensity and photoperiod (Kolderup 1975; Spiertz 1977; Van Herwaarden et al. 1998). Genotypic differences have also been reported for traits influencing GPC, including pre- and post-anthesis N-uptake (Woodruff 1972; Austin et al. 1977; Cox et al. 1985a) and nitrogen harvest index (Flood and Martin 2001).

Interpreting G × E effects on GPC requires understanding of the dynamics of supply and demand relations (the source/sink balance), with the relation between C and N being of special interest. Because C assimilation increases faster than N accumulation, GPC usually decreases with greater grain yield (e.g. Lawlor 2002). Grain N accumulation is mostly source-driven and is determined by N stored in vegetative organs and N-uptake from soil during the post anthesis period. Grain yield is mainly determined during pre-anthesis growth but is also influenced by the duration after anthesis (Richards 2000; Martre et al. 2003; Slafer 2003).

Acuña et al. (2005) showed that a shorter pre-anthesis phase (induced by exposition to long days), resulted in low grain yield and increased GPC. Early anthesis, long grain filling duration, low grain filling rate, and high protein concentration were strongly interrelated in F_1 hybrids of Chinese and U.S. cultivar diallel crosses (Mou et al. 1994). Since date of anthesis is highly heritable (e.g. Hsu and Walton 1970), analyses of variation in GPC might benefit from considering the influence of pre-anthesis development. Emphasis on optimizing phenology has special relevance given growing concerns over climatic risk and global warming, which may induce wheat producers to seek earlier maturing cultivars to reduce risk or conversely to seek cultivars with longer growth cycles to exploit longer growing seasons.

Cultivar differences in pre-anthesis development are mainly determined by vernalization requirement, photoperiod sensitivity and earliness per se. Many reports have provided semi-quantitative descriptions for cultivar differences in these traits, but in most cases, responses were determined using artificial environments

(e.g. Davidson et al. 1985; Hoogendoorn 1985; Kato et al. 2001), and the results appear to be difficult to use for quantitative predictions of phenology. Promising alternatives include characterizing cultivar differences through parameters used in process-based ecophysiological models (Herndl et al. 2008) and using genetic data to characterize these three traits (White et al. 2008). Used as inputs to a process-based model, such parameters can reliably predict crop phenology. Since simulation models can integrate interacting effects of genotypic differences, temperature, photoperiod regimes, and management (mainly time of planting), it seems likely that model-based characterizations of cultivars can help explain G × E interactions for GPC. Another option would be to analyze simulated variation in GPC. However, simulations require detailed characterizations of initial soil nitrogen and organic matter status and of crop management, and such data are seldom available for wheat breeding nurseries.

The objectives of this paper are to test whether vernalization requirement, photoperiod response and earliness per se partially explain cultivar differences in GPC and to examine whether including effects of the three traits are useful in analyzing G × E interactions for GPC. Two contrasting sets of data were considered, one from a widely distributed set of international cultivar trials and one from a single location that involved detailed characterizations of cultivar responses.

The research was anticipated to require consideration of processes that interact strongly along causal paths. Path analysis can be used to test schematic models that specify causal relations among multiple variables. The procedure determines how well a proposed model successfully accounts for the actual relationships observed in the sample data (Hatcher 1994). For agronomic purposes, path analysis is often used to assist identifying selectable traits (Duarte and Adams 1972; Kang et al. 1983). To use path analysis for investigation of causal relations among traits influencing wheat phenology and GPC, a theoretical model must be specified first. Causal relations can be based on experimental results or on a priori grounds. Figure 1 presents a simple model of a possible cause and effect relationship between phenological traits and GPC. The single-headed straight arrows represent direct effects between endogenous variables (variables where the variability is predicted to be causally affected by other variables in the model) and

Fig. 1 The initial theoretical model (cause and effect relationship) among phenological traits and grain protein content

ª Days from planting
E Error term
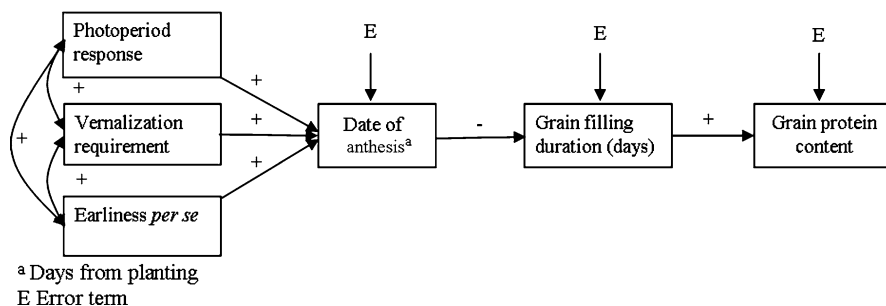
exogenous variables (constructs that are influenced only by variables that are outside the model). The double-headed curved arrows indicate correlations among exogenous variables.

Materials and methods

IWWPN trials

Data for GPC evaluated for a range of sites, years, and cultivars were obtained from digitized reports of the International Winter Wheat Performance Nurseries (IWWPN) stored at CIMMYT (IWIS; Payne et al. 2002). These series of trials were organized by the Nebraska Agricultural Experiment Station and the Agricultural Research Service, U.S. Department of Agriculture, under a contract with the Agency for International Development. The nurseries were distributed in over 40 countries. Altogether 168 wheat cultivars and breeding lines were tested from 1969 to 1981, the vast majority being winter types. Nurseries contained 30 entries per year and at each site, were arranged in four randomized complete blocks. Agronomic practices varied with site but most often were representative of nearby commercial production. Precipitation ranged from a high of 1,804 mm at Morioka, Japan in 1981 to a low of 22 mm at Toluca, Mexico in 1973. Supplemental irrigation was applied in a few nurseries (4–11 nurseries, depending on the year). Nitrogen fertilizer was applied to most nurseries, with rates ranging from 5 to 224 kg N ha^{-1}. GPC was determined at a single laboratory in Nebraska.

Twenty-four winter wheat and five spring wheat cultivars were selected based on available data for GPC (Table 1). The cultivars had been tested at 55 sites in 32 countries over 13 years (Table 2).

To simplify the interpretation of G × E variance components, locations were classified in seven regions according to the classification of Peterson and Pfeiffer

(1989) (Table 2). Locations associated with Region 1 tend to have low potential grain yield and due to the continental climate influence, winter stress affects cultivar adaptation. Region 2 locations have later heading and ripening dates, associated with higher grain yield potential, and they predominantly include locations from Western and Central Europe with a maritime climate. Regions 3 and 4 tend towards earlier heading and ripening dates and are centered in the Middle East and Eastern Europe, respectively. Region 5 has later heading and ripening dates. Region 6 includes three sites form South America, and Region 7, two sites from South Africa. These latter two regions have mild winters and are mainly considered as spring and facultative wheat regions.

Field experiments at Ihinger Hof

During the growing seasons of 2004–2005 and 2005–2006, field experiments with 12 winter wheat cultivars were conducted at Ihinger Hof, Germany (48°44′ N; 8°56′ E; 450 m elevation, 693 mm average annual precipitation, 8.1°C mean annual temperature) to provide data on crop growth and development. According to WRB-classification (FAO 1998), the soil type is a Haplic Luvisol. The texture ranges between silty clay and clayey silt. Seed was planted at October 23, 2004 and October 12, 2005 at a rate of 350 kernel m^{-2} in plots consisting of 12 rows, 14 cm apart and 6 m long. Nitrogen was applied as ammonium sulphate at the rate of 50 kg N ha^{-1} at the beginning of vegetative growth and as calcium ammonium nitrate at the rate of 25 kg N ha^{-1} at Zadoks stage 30 in 2004. In 2005, nitrogen was applied using the same fertilizer and stages at rates of 35 and 20 kg N ha^{-1}, respectively. Together with mineralized nitrogen (assessed before the first nitrogen application), the plants received approximately 100 kg N ha^{-1} in each trial. The experiment was arranged as a split plot

Table 1 Cultivar parameters P1V (days at optimum vernalizing temperature required to compete vernalization), P1D (percentage reduction in development rate in a photoperiod 10 h shorter than the optimum relative to the rate at the optimum) and calibrated sum of the component phase durations (P_{123}) of cultivars calibrated over 2 years at the research station Ihinger Hof, Germany and IWWPN cultivars calibrated over 55 sites and 13 years (Table 2)

Cultivar	Origin[a]	Habit	Cultivar parameters		
			P1V (days)	P1D (%)	P_{123} (°C days)
Aurora	USSR	WW	68	24	810
Balkan	Yugoslavia	WW	69	25	760
Bastion	Netherlands	SW	59	46	760
Bezostaya 1	USSR	WW	60	31	760
Biserka	Yugoslavia	WW	54	29	750
Bounty	England	WW	58	45	800
Bussard[b]	Germany	WW	55	61	740
Capppell Desprez	France	WW	68	38	810
Contra[b]	Germany	WW	56	55	760
Dream[b]	Germany	WW	48	63	760
Drifter[b]	Germany	WW	59	58	760
Dwarf Bezostaya	USSR	WW	68	25	780
Enorm[b]	Germany	WW	39	52	750
Excellent[b]	Germany	WW	45	58	740
Hybnos 2b[b]	Germany	WW	50	59	735
INIA 66	Mexico	SW	17	30	760
Irnerio	Italy	SW	47	32	780
Jugoslavija	Yugoslavia	WW	58	34	765
Lerma Rojo 64	Mexico	SW	31	39	740
Mandub[b]	Germany	WW	47	61	760
Maris Huntsman	England	WW	64	47	760
Maris Mardler	England	WW	57	43	800
Maris Nimrod	England	WW	71	37	805
Maris Templar	England	WW	77	37	790
Mironovskaya 808	USSR	WW	69	34	755
Moslavka	Yugoslavia	WW	55	20	775
Odesskaya 51	USSR	WW	61	19	790
Opus[b]	Germany	WW	59	59	740
Partizanka	Yugoslavia	WW	64	15	760
Phoenix	Australia	WW	52	16	810
Renan[b]	France	WW	44	54	740
San Pastore	Italy	WW	47	18	745
Sanja	Yugoslavia	WW	57	32	760
Sava	Yugoslavia	WW	39	40	755
Super X	Mexico	SW	49	33	765
Talent	France	WW	58	29	770
Terrier[b]	Germany	WW	61	59	750

Table 1 continued

Cultivar	Origin[a]	Habit	Cultivar parameters		
			P1V (days)	P1D (%)	P_{123} (°C days)
Tiger[b]	Germany	WW	51	57	740
Vakka	Finland	WW	55	43	780
Zlatna dolina	Yugoslavia	WW	62	24	770
Zlatoklasa	Yugoslavia	WW	56	24	770

Habit is as reported with SW = spring and WW = winter

[a] Origin of the non-German cultivars as reported in the International Winter Wheat Performance Nursery (e.g. Kuhr et al. 1984)

[b] Cultivars evaluated in the study conducted at the research station Ihinger Hof 2004/2005 and 2005/2006

design with three replicates, with cultivars as the main plots.

Developmental stages were assessed visually, based on when 50% of plants reached a given stage. At maturity, 1 m² from each plot was harvested to provide seed for the GPC analyses. Nitrogen content of grain was determined by the micro-Kjeldahl method (Bradstreet 1965). From this, GPC was calculated by the conversion factor 5.7 for winter wheat (bread) (Sosulski and Imafidon 1990). Durations from planting until date of anthesis and until physiological maturity were calculated from daily mean temperatures as growing degree days (°C days) assuming a base temperature of 0°C.

Cultivar parameters

Cultivar differences for the 12 cultivars used in the field study and the 29 cultivars selected from the IWWPN trials were characterized using the model parameters P1V for vernalization requirement, P1D for photoperiod response, and P1, P2 and P3 for earliness per se from CSM-Cropsim-CERES-Wheat model Version 4.0.2.0 (Jones et al. 2003; Hoogenboom et al. 2004). The three coefficients affecting earliness per se were summed to provide the factor P_{123} with units of °C days. P1V, P1D and P1 were estimated using the GenCalc2 software, which facilitates testing ranges of parameter values and estimating goodness of fit (Hunt et al. 1993). Calibrations for the IWWPN entries were done on the subset of nurseries used by White et al. (2008), representing approximately one third of the locations and data available for those cultivars. P2 and P3 were held constant during the calibration. The cardinal temperatures for

Table 2 Countries, site, latitude, number of cultivars, years and experiments, selected for the analysis of the impact of E, G, G × E and the influence of the cultivar parameters as a co-variable on grain protein content

Country	Site	Latitude	Number of cultivars	Number of years	Number of experiments
Region 1					
Finland	Jokioinen	60.49	21	7	34
Japan	Morioka Iwate	39.45	20	9	50
Norway	Vollebekk	60.00	11	6	19
South Korea	Suwon	36.19	22	10	49
Turkey	Ankara	40.00	19	9	55
Ukraine	Mironovski	50.15	6	3	11
	Odessa	46.00	22	6	40
United States	Akron	40.10	7	3	12
	Brookston	40.25	6	2	8
	Fort Collins	40.35	29	11	70
	Hutchinson	38.00	6	3	11
	Ithaca	42.30	29	11	63
	Lincoln	40.50	20	9	40
	Rowan Co.	35.42	29	13	70
	Stillwater	36.07	29	12	66
Region 2					
Austria	Vienna	48.12	29	11	68
Croatia	Zagreb	45.49	22	10	59
France	Orgerus	48.50	3	1	3
Germany	Langenstein	51.42	19	5	30
	Monsheim	49.35	29	12	72
	Weihenstephan	48.24	29	11	68
Netherlands	Wageningen	51.28	21	11	63
Poland	Przeclaw	50.18	15	5	27
	Warsaw	52.12	26	7	49
United States	Corvallis	44.30	24	6	42
United States	Pullman	46.42	27	10	90
Slovakia	Bratislava	48.29	26	7	46
Sweden	Svalof	55.35	22	11	92
Switzerland	Zurich	47.29	29	11	69
Region 3					
Iran	Karaj	50.35	21	10	58
Iraq	Sulaimaniya	36.30	19	8	50
Italy	Milano	45.13	29	11	72
	Rieti	42.24	29	11	63
Afghanistan	Heart	34.11	12	3	17
	Kabul	34.27	15	8	49
Argentina	Bordenave	−37.51	29	12	78
Bulgaria	Tolbukhin	43.40	20	9	54
Russia	Krasnodar	45.00	26	9	61
Syria	Aleppo	36.05	7	2	10
United States	Davis	38.32	29	9	53

Table 2 continued

Country	Site	Latitude	Number of cultivars	Number of years	Number of experiments
Region 4					
Hungary	Martonvasar	47.21	20	9	56
	Szeged	46.00	19	8	49
Romania	Fundulea	44.03	29	12	73
Spain	Madrid	40.31	14	2	15
Serbia Montenegro	Novi Sad	45.30	29	12	77
Region 5					
Czech Republic	Sedlec	50.14	26	8	51
Turkey	Erzurum	39.58	26	8	52
	Eskisehir	36.45	21	10	61
United States	Billings	45.00	19	6	34
Iran	Hamadan	34.47	18	7	42
Region 6					
Argentina	Balcarce	−37.45	20	7	41
Chile	Chillan	−36.31	15	4	24
	Temuco	−38.40	22	10	58
Region 7					
South Africa	Bethlehem	−28.10	24	7	67
Mexico	Toluca	19.16	26	8	59

Region classification was based on Peterson and Pfeiffer (1989)

vernalization were modified from values of −5, 0, 7, and 15°C (Hoogenboom et al. 2004) to −4, 0, 3, and 15°C as suggested by White et al. (2008).

Path analysis to test the relationship between phenological traits and grain protein content

Path analysis was performed to test the theoretical model (Fig. 1) with data obtained from the field experiments at Ihinger Hof. Goodness of fit was estimated using two parameters. The *P*-value associated with the model χ^2 test and the non-normed fit index (NNFI; Bentler and Bonnet 1980) that represents the percentage of observed-measure covariation explained by a given structural model as compared with an overall, null model. To indicate acceptable agreement between modeled and observed values, the *P*-value associated with the model χ^2 test should exceed 0.05, the NNFI index should be over 0.9, and all path coefficients should be significant at the $P = 0.05$ level.

Before using the iterative approach of Hatcher (1994) to assess the fit between model and data, the causal relationships were reviewed to see whether causal relations could be improved by modifying the parameter set. This iterative approach consisted of reviewing residual normalized residual matrix, χ^2 test,

NNFI and significance tests for path coefficients. If a path did not improve model and data fit, it was deleted. If an alternative path improved the model, that path was added, and the revised model was re-estimated.

Statistical analyses

Statistical analyses were conducted using procedures of the SAS program (SAS Institute 2000). The MIXED procedure was used to evaluate the relative impacts of E, G, G × E on GPC by the determination of variance components and to conduct a covariance analysis to test the influence of the model parameters on GPC with the IWWPN dataset. Path analyses were conducted using the CALIS procedure with the maximum likelihood method of parameter estimation. These analyses were performed on a variance-covariance matrix.

Results

Importance of genotype, environment and their interactions on grain protein content

The estimated variance components of GPC for the IWWPN dataset showed large effects of region ×

site × year and regions × site (Table 3). Cultivar differences explained 7% of the total variance for GPC as compared to 41% explained by region ×

Table 3 Estimated variance components for cultivar, region, site and year on grain protein content

Covariance parameter	Variance component	% of total variance	Standard error	P-value
Cultivar	0.31	7	0.10	0.001
Region	0.31	7	0.26	0.113
Region × cultivar	0.08	2	0.03	0.003
Region × site × cultivar	0.19	4	0.03	<0.001
Region × year × cultivar	0.05	1	0.02	0.006
Region × site	0.80	18	0.22	<0.001
Region × year	0.09	2	0.08	0.129
Region × site × year	1.85	41	0.16	<0.001
Residual	0.85	19	0.04	<0.001

Data were for a subset of IWWPN trials (e.g. Kuhr et al. 1984) and included 5 spring and 24 winter wheat cultivars, 7 regions, 55 sites and 13 years (Tables 1 and 2)

site × year and 18% by regions × site. The remaining variation of 34% was explained by region (7%), region × cultivar (2%), region × year (2%), region × site × cultivar (4%), and regions × year × cultivar (1%), leaving a residual variation of 19%.

The covariance analysis indicated that the effects of P1V, P1D and P_{123} on GPC differed with growth habit (Table 4). When spring and winter wheats were examined together, effects of P1V, P1D and P_{123} on GPC varied with region, site and year. When the analysis was restricted to the 24 winter wheats, the effects of region, site and year were still observed for P1V but not for P1D and P_{123}. Covariance analysis for the five spring wheats detected no effect of P1V, P1D and P_{123} on GPC.

To analyze location-specific responses of P1V, P1D and P_{123} on GPC, the effects were estimated within regions, and locations were arranged and displayed according to their latitude (Fig. 2). The effect of P1V on GPC declined at higher latitudes in Regions 2 and 4 (Fig. 2a). The effect of P1D on GPC decreased with latitude in Region 3 and 4 (Fig. 2b), and in Regions 2 and 4, the effect of P_{123} also decreased with latitude (Fig. 2c).

Table 4 Covariance analysis for effects of the cultivar parameters P1V (days at optimum vernalizing temperature required to compete vernalization), P1D (percentage reduction in

development rate in a photoperiod 10 h shorter than the optimum relative to that at the optimum) and calibrated sum of the component phase durations (P_{123}) on grain protein content

Effect	Habit								
	DF			F-value			P-value		
	SW	WW	SW + WW	SW	WW	SW + WW	SW	WW	SW + WW
P1V	1	1	1	0.00	0.03	3.66	0.999	0.872	0.571
P1V × region	6	6	6	0.00	0.19	1.38	1.000	0.979	0.222
P1V × region × site	46	48	48	0.39	14.01	47.28	0.968	<0.001	<0.001
P1V × region × year	77	77	77	0.00	0.14	16.72	1.000	1.000	<0.001
P1V × region × site × year	233	313	314	1.32	12.90	14.42	0.396	<0.001	<0.001
P1D	1	1	1	0.00	0.04	0.03	0.999	0.8362	0.869
P1D × region	6	6	6	0.00	0.50	0.50	1.000	0.8075	0.136
P1D × region × site	30	48	48	0.44	0.86	2.35	0.938	0.7269	<0.001
P1D × region × year	15	72	77	0.00	0.59	1.95	1.000	0.9926	<0.001
P1D × region × site × year	14	310	312	0.61	1.09	1.56	0.789	0.2944	<0.001
P_{123}	1	1	1	0.00	0.04	0.49	1.000	0.8472	0.483
P_{123} × region	5	6	6	0.00	1.41	1.47	1.000	0.2152	0.189
P_{123} × region × site	22	47	48	0.47	1.90	4.01	0.910	0.0026	<0.001
P_{123} × region × year	5	65	77	0.00	0.81	2.09	1.000	0.8269	<0.001
P_{123} × region × site × year	2	271	299	0.11	1.43	1.68	0.897	0.0116	<0.001

Data were for a subset of IWWPN trials (e.g. Kuhr et al. 1984) and included 5 spring (SW) and 24 winter wheat (WW) cultivars, 7 regions containing 55 sites, and 13 years of evaluations (Tables 1 and 2)
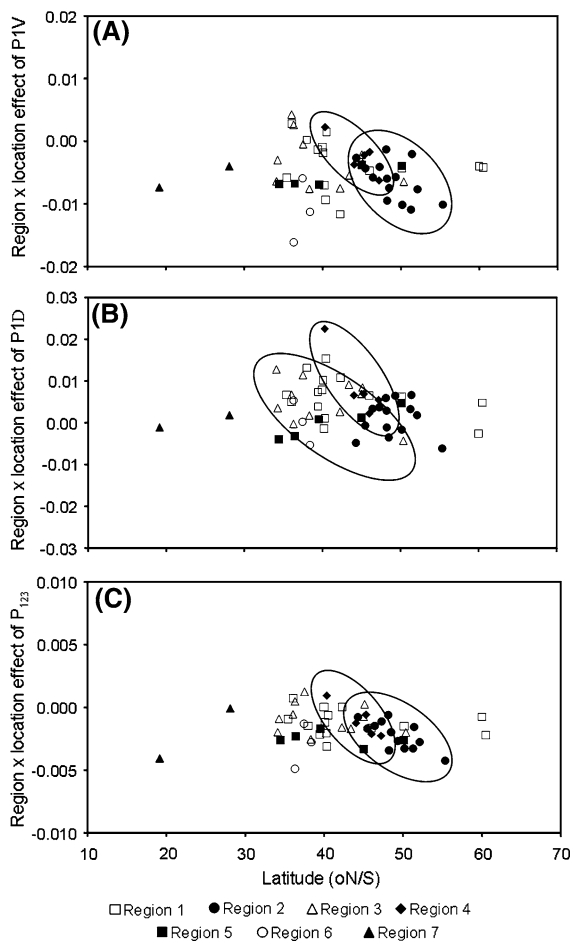
Fig. 2 Relations between location effect of model parameters P1V, P1D and P_{123} on GPC and absolute latitude of 5 spring and 24 winter wheat cultivars tested in the IWWPN dataset. (**a**) P1V, (**b**) P1D, and (**c**) P_{123}. The ellipses indicate specific regions showing strong relations between effects and latitude

Path analysis of the traits measured in Germany

Weather conditions during the two experiments in Germany were similar (Table 5). Seasonal patterns for air temperature during 2004–2006 were almost the same, and precipitation in both growing seasons was adequate to avoid water deficits. The high rainfall in August 2006 delayed maturity, but over all, the two seasons resulted in similar responses for phenology and GPC.

The initial path model using days to anthesis and maturity for 12 winter cultivars evaluated over two seasons showed poor agreement with observed data (Table 6), so the model was revised to use thermal time (Model 1; Table 7). This improved the goodness of fit, but the results were still judged unacceptable (Table 7). Thus, Model 1 was rejected, and an attempt was made to identify a better model by reviewing whether any of the paths in Model 1 should be deleted. The t values for the path between P1V and thermal time until anthesis and thermal time anthesis and grain filling duration were not significant ($P < 0.05$), so their paths were excluded. The resulting Model 2 was then re-estimated and found to provide a good fit to the data (Table 7), with all path coefficients significant at $P < 0.05$ (Fig. 3a).

To examine whether Model 2 was sensitive to year of evaluation, Model 2a was estimated using data only from the year 2005. Goodness of fit indices from Model 2 and Model 2a were comparable (Table 7).

In the path analyses with Model 2 (Fig. 3a), thermal time to anthesis had a negative direct effect (path coefficient $pc = -0.66$; $P < 0.001$), whereas grain filling

Table 5 Monthly mean temperature and precipitation during the growing seasons in 2004/2005 and 2005/2006 at the research station Ihinger Hof, Germany

Data	Growing season	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Mean temperature, °C	2004/2005	11	4	0	1	−2	4	9	13	18	18	15
	2005/2006	11	4	0	−3	0	2	8	13	17	22	14
	Mean for 1977–2004	9	3	1	−1	0	4	7	12	15	17	17
Precipitation, mm	2004/2005	88	28	40	34	42	60	58	73	44	85	80
	2005/2006	32	26	29	11	26	60	63	69	33	57	138
	Mean for 1977–2004	60	51	54	44	40	47	50	82	78	73	64

Table 6 Relationships between pairs of variables for 2005 data (above the diagonal) and for combined 2005 and 2006 data (below the diagonal) of 12 winter wheat cultivars tested at the research station Ihinger Hof, Germany

	Time to anthesis (days)	Thermal time till anthesis (°C days)	Grain filling duration (days)	Grain filling duration (°C days)	P1V	P1D	P_{123}	GPC
Time to anthesis (days)	–	0.99***	−0.09	−0.17	0.31	0.88***	0.48	−0.58*
Thermal time till anthesis (°C days)	0.42*	–	−0.09	−0.17	0.30	0.88***	0.49	−0.58*
Grain filling duration (days)	−0.52**	−0.03	–	0.99***	−0.42	−0.03	−0.14	0.15
Grain filling duration (°C days)	0.69***	0.29	0.22	–	−0.45	−0.12	−0.15	0.21
P1V	0.08	0.32	−0.15	−0.07	–	0.38	0.12	−0.48
P1D	0.18	0.72***	0.24	0.28	0.38	–	0.12	−0.56
P_{123}	0.13	0.53**	−0.01	0.08	0.12	0.12	–	−0.14
GPC	0.02	−0.59**	−0.09	0.05	−0.44*	−0.41*	−0.31	–

*, **, *** Significant at $P < 0.05$, <0.01 and <0.001, respectively

Table 7 Goodness of fit indices for the path models

Model	χ^2	DF	P-value	Non-normed fit index
Initial model	15.773	7	0.03	−0.401
Model 1[a]	15.413	7	0.03	0.503
Model 2[b]	0.504	3	0.92	1.243
Model 2a[c]	0.579	3	0.90	1.347

[a] Identical to the initial model (Fig. 1), except using thermal time for the duration from planting till anthesis and anthesis to physiological maturity

[b] Identical to the Model 1, the path between P1V and thermal time till anthesis and the path between thermal time till anthesis and grain filling duration were deleted

[c] Identical to the Model 2, but using only data from the year 2005

duration showed a positive direct effect ($pc = 0.25$; $P < 0.05$) on GPC. Direct effects on thermal time until anthesis were −0.67 for P1D ($P < 0.001$) and 0.45 for EPS ($P < 0.001$). Results for 2005 were comparable with results obtained using both years (Fig. 3b). Path coefficients of second-order variables on GPC were obtained by calculating the product of the intermediate path coefficients within the path to GPC (Table 8). The path coefficients for P1D and P_{123} on GPC were −0.44 and −0.30, respectively.

Discussion

The IWWPN provided data for a widely distributed and diverse set of germplasm and environments,

including differences in altitude, latitude, climate and management. Peterson and Pfeiffer (1989) proposed that long-term performance nursery data allow more precise definition of site relationships because the multiple years of observation minimize the effect of
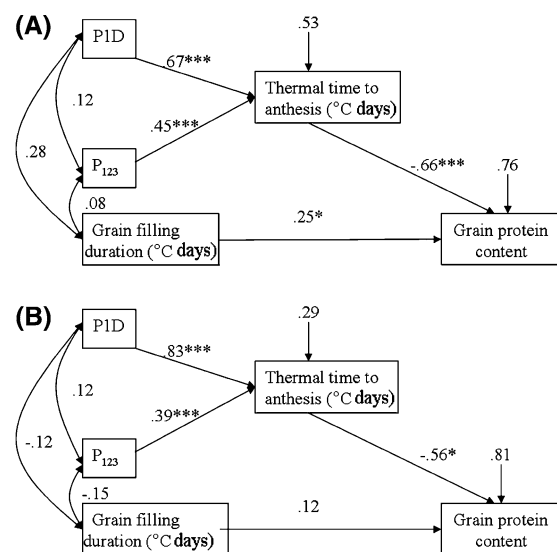
Fig. 3 Path diagram for 12 cultivars tested at Ihinger Hof, Germany. Standardized path coefficients appear on single-headed straight arrows, and correlations appear on double-headed curved arrows. (**a**) Model 2 (identical to the initial model, except that the path between P1V and thermal time until anthesis and the path between thermal time until anthesis and grain filling duration were deleted) applied to data from 2005 and 2006. (**b**) Model 2a. Path diagram as in (**a**) except restricted to 2005. *, **, *** Significant at $P < 0.05$, <0.01 and <0.001, respectively

Table 8 Path coefficients of phenological traits on grain protein content and traits along its path to grain protein content

Exogenous variable	Path coefficients	
	Thermal time till anthesis (°C days)	Grain protein content
Grain filling duration (°C days)		0.25
Thermal time till anthesis (°C days)		−0.66
P1D	0.67	−0.44
P_{123}	0.45	−0.30

Data are for 12 winter wheat cultivars tested at the research station Ihinger Hof, Germany in 2005 and 2006

unusual or short term weather patterns. The variance components estimated for the impact of genotype, environment and their interactions on GPC confirmed that, as expected, environment had a major influence on GPC, but differences among genotypes were still readily detectable (Table 3). The covariance analyses showed that the cultivar parameters P1V, P1D and P_{123} partially explained cultivar variation in GPC (Table 4), but further indicated that the effects varied with site and year within region. When only winter wheats were considered, the effect of P1V was similar to the combined analysis but not for P1D and P_{123}.

The analysis of location effects of P1V versus latitude (Fig. 2) emphasized that relationships between GPC and the three phenological traits varied with environment. For regions where strong relations with latitude were found (e.g. Region 4 for all three traits), the effects decreased with latitude. This agrees with the expectation that at high latitudes, temperatures are cool enough to ensure full vernalization and photoperiods are long enough to minimize effects of photoperiod. We note, however, that the impact of these traits on GPC requires detailed analysis for each region. Ideally, this would include use of datasets where soil nitrogen status is characterized in order to allow application of simulation models.

Path analyses for Ihinger Hof confirmed that GPC increases with a shorter pre-anthesis phase (e.g. Mou et al. 1994; Le Gouis et al. 2000; Talbert et al. 2001). A direct effect from time to anthesis on grain filling duration was not detected, but grain filling duration affected GPC, as observed by Knott and Gebeyehou

(1987) for durum wheat. In the Ihinger Hof dataset, P1D and P_{123} had negative effects on GPC (Table 8). For P1V, the relation obtained in the co-variance analysis was not confirmed by the path analyses. The absence of an effect of P1V at this location likely was due to all cultivars being completely vernalized.

The foremost implication of this work is that even for traits such as GPC that appear to be relatively remote from pre-anthesis development, vernalization requirement, photoperiod sensitivity and EPS may still have important influences. For GPC, research should identify combinations of these traits that are best suited for their target population of environments. The genetic control of the traits is relatively well understood (e.g. Laurie et al. 2004; White et al. 2008), and recent progress in sequencing the *Vrn* and *Ppd* loci gives promise that genetic information may become readily available for many cultivars (e.g. Sherman et al. 2004; Beales et al. 2007). This would further facilitate targeting of germplasm to environments, as proposed by Kato et al. (2001) for additive effects of *Vrn-1* loci.

Direct simulation of effects of cultivar traits on GPC is another promising option (Martre et al. 2006), but requires greater attention to characterization of soil conditions and crop management. Models integrate specific hypotheses about interacting processes and thus beyond their use as predictive tools, have utility in guiding additional research.

Conclusions

Analyses at contrasting geographic scales confirmed that cultivar differences in GPC can be influenced by vernalization requirement, photoperiod response and earliness per se. An immediate practical application of these findings is that attempts to analyze cultivar differences in grain nitrogen should consider the influence of simply inherited traits that help determine the duration of vegetative growth. The work also provides a foundation for more explicit consideration of how altering crop phenology in response to climate risk or global change would affect environmentally sensitive traits such as GPC.

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